

# Recurrent Selection to Alter Grain Methionine Concentration and Improve Nutritional Value of Maize

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## ABSTRACT

Methionine is an essential amino acid that is limiting in maize- (*Zea mays* L.) based diets. The objective of this work was to determine whether we could alter grain methionine concentration in random-mated maize populations by mass selection for methionine concentration using a microbial assay. In one study, we developed two populations by selecting for high or low methionine concentration (HM or LM, respectively) for three generations starting from the random-mated population BS11. Grain from these populations was used to formulate diets for a feeding trial in which 15 rats were fed HM grain and 15 rats were fed LM grain. Rats on the HM diet had a 0.018 higher feed efficiency (g gain/g feed) than rats on the LM diet. In a second study, we performed three cycles of selection for high or low methionine concentration starting with two random-mated populations, BS11 and BS31. We evaluated each cycle of selection in a field trial with two replications in each of two years. Methionine concentration was significantly correlated with the cycle of selection, changing on average 0.004 g methionine/100 g grain per cycle. Kernel mass, %N, oil, protein, starch, tryptophan, and lysine concentration did not exhibit significant correlations with cycle of selection. We conclude that recurrent selection for grain methionine concentration using a microbial assay is an effective method to alter methionine content.

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**Abbreviations:** HM, high methionine; LM, low methionine; QPM, quality protein maize.

**I**N MEAT PRODUCTION, the greatest expense is the cost of feed. Dietary improvements that result in decreased feed consumption per weight gain would therefore reduce the cost of meat production. Maize is often a major component of animal diets, and is therefore an attractive target for modifications that improve its nutritional value. Maize is nutritionally limited by deficiencies in lysine, methionine, and tryptophan. Feed methionine concentration is particularly problematic when maize is supplemented with legume protein, which is also deficient in methionine. These deficiencies can be corrected by supplementation, although this adds to per head production costs. Genetic improvements resulting in increased levels of these amino acids would be valuable because they would reduce the amount of supplementation required.

Mutation breeding has been shown to be an effective method for improving the amino acid balance of maize. Maize with improved lysine and tryptophan concentration has been developed using the *opaque2* mutation and is called QPM (quality protein maize) (Vasal, 2001). A mutation affecting methionine concentration was discovered in a screen for lysine + threonine resistant maize seedlings (Phillips and McClure, 1985). While the genetics of this mutation (designated *dzt1*) are complicated (Chaudhuri and Messing, 1994), it has been used successfully to

Published in Crop Sci. 48:1705–1713 (2008).

doi: 10.2135/cropsci2008.01.0010

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increase the methionine concentration of maize hybrids (Olsen et al., 2003).

Recurrent selection is an attractive method for controlling quantitative traits because it only requires a method to measure the trait and germplasm with heritable variation for the trait. Grain composition has been successfully manipulated using recurrent selection. The Illinois long-term selection program has resulted in populations containing the known extremes of protein concentration in maize (Dudley and Lambert, 2004). These populations have proven valuable for understanding the physiology of traits related to grain protein concentration (Below et al., 2004). Recurrent selection for grain amino acid concentration has not been used extensively in spite of the fact that it has been shown to be successful for increasing lysine concentration (Choe et al., 1976). One reason recurrent selection for methionine has traditionally not been used extensively is the amount of labor and expense involved in measuring methionine concentration by HPLC. However, microbial methods are inexpensive, adaptable to high-throughput analysis and have been shown to be effective for grain analysis (Wright and Orman, 1995). High throughput microbial methods have been used for the analysis of commercial maize hybrids (Darrigues et al., 2005) and germplasm from a QPM breeding program (Scott et al., 2004).

The objective of this study was to determine the effect on nutritional value and grain composition of selection for grain methionine concentration in maize. We conducted two experiments in which we developed maize populations by recurrent selection for either high or low grain methionine concentration. The goal of the first experiment was to compare the feed efficiency of grain selected for high or low methionine concentration using a rat feeding study. The goal of the second experiment was to evaluate plant and grain traits in populations derived from three cycles of divergent selection for or against methionine.

## MATERIALS AND METHODS

### Populations Used in This Study

Two different maize populations were used in this study. One population was derived from BS11, a population originally designated as "Pioneer Two-ear Composite". It was developed by crossing southern prolific material and corn belt lines (Hallauer, 1967). The second population was derived from BS31 population, another open-pollinated synthetic population derived from FS8A(T)C4. The FS8A population was initially developed at the Florida Agricultural Experiment Stations and released in 1988. Germplasm from southeastern U.S., corn belt, and tropical sources, respectively, account for approximately 30, 22, and 48% of FS8A(T). The initial objective in the development of this population consisted of intermating a wide range of accessions for resistance to southern corn leaf blight (Horner, 1990). The BS11 and BS31 material used in this study has been under selection for agronomic performance for multiple cycles of recurrent selection.

## Breeding Strategy

One hundred and two hundred half-sib ears from the populations BS11 and BS31, respectively, were produced in the summer of 2000, analyzed, and categorized on the basis of their methionine and tryptophan concentration. The ears with the five highest and five lowest concentrations for each amino acid were selected from each population, giving four categories each containing five selected ears. These categories were called BS11HM, BS11LM, BS31HM, and BS31LM. Thus, the BS11HM category represents the ears from the BS11 population with the highest concentration of methionine (HM), while BS31LM represents the ears from BS31 with the lowest concentration of methionine, and so on.

To generate grain for the feeding trial, seed of the selected ears from all four categories was planted in the summer of 2001 at the Iowa State University Agronomy Farm located in Boone, Iowa. Two adjacent rows of each selection were planted, representing a total of 80 rows, and the plants *within* each selection were intermated in a chain-sib mating design. The ears were harvested individually and analyzed for concentration of methionine. Five ears were selected from each category as either the highest or the lowest ears depending on the category. Three adjacent rows of 25 kernels were planted in 2002 for each selection and the plants were intermated *within* each selection in a chain-sib mating design.

Approximately 30 ears from each selection were harvested in a bulk for a total of about 20 bulks, 10 from each starting population (BS11 or BS31) with five HM selections and five LM selections. The methionine concentration of each bulk was analyzed using the microbial assay (Darrigues et al., 2005), and three bulks were selected to represent the HM and LM categories of each population. A subsample of the bulks derived from BS11 representing each category was sent to the University of Missouri-Columbia Experiment Station Chemical Laboratories for a complete amino acid analysis by ion exchange chromatography according to the AOAC official method 982.30 E (a,b,c). Also, NIR spectroscopy was used to predict the protein, oil and starch concentration of each bulk using a Foss 6500 spectrophotometer (Foss NIR Systems, Inc., Laurel MD). These bulks were used to formulate the diet in the feeding trial.

The recurrent selection experiment started with the same four populations used in the breeding program to generate the feeding trial diets. In this program, however, intermating was performed *among* the selections made in 2000 in the following manner. For each category (e.g. BS31HM), a balanced bulk was made with each of the selections within the category. The four bulks were then planted in adjacent rows of 25 kernels. The plants in each bulk were intermated in a chain-sib mating design. Resulting ears were harvested individually and their methionine concentration was analyzed. Five selections from approximately 40 ears in each category were chosen on the basis of their amino acid concentration as before. These selections were used to make balanced bulks that constitute the Cycle 1 population. Two more cycles of selection were performed similarly with 50 ears being evaluated and the five ears highest (for the HM populations) or lowest (for the LM population) for grain methionine concentration were selected in each cycle.

## Evaluation of Three Cycles of Selection for High or Low Methionine Concentration

Twelve entries (BS11HM cycles 1–3, BS11LM cycles 1–3, BS31HM cycles 1–3, and BS31LM cycles 1–3) were produced in a field experiment using a randomized complete block design with replicates comprising two blocks. The experiment was grown at the Iowa State University Agronomy Farm near Boone, Iowa in 2004 and 2005. Each treatment in the experiment consisted of four adjacent 5.5 m rows spaced 1 m apart and planted with 25 seeds each. Plants were allowed to pollinate naturally. Days to silking and days to flowering were determined by noting the number of days after planting that 50% of the plants in the middle two rows of the four row plots reached silk emergence or pollen shed, respectively. Plant height was measured after pollen shed was complete by measuring the height of the second through the sixth plants in each of the middle two rows and averaging the resulting 10 values. Ear height was measured the same way except the height of node at which the ear shank attached to the stalk was noted. When grain reached approximately 18% moisture, five ears were harvested from the middle two rows of each plot. Two flat kernels from the center of each ear were randomly selected and bulked to represent the plot. Grain samples were dried to about 12% moisture using forced air. Protein, oil, and starch concentration were predicted by NIR. Grain samples were ground and %N was determined by combustion analysis at the Iowa State University Soil and Plant Testing Laboratory using a Leco TruSpec CN (St. Joseph, MI). Methionine concentrations were determined using the microbial assay described below. In addition, amino acid analyses of the cycle 3 samples (HM and LM) were performed at the University of Missouri Experiment Station Chemistry Lab using the AOAC standard method (AOAC International, 1995a,b).

## Microbial Analysis of Methionine Levels

From each experiment, each ear of maize was shelled and packaged individually. From each ear, five random whole kernels were ground to a fine powder using a Wiley Mill with a 40-mesh screen. The powder of each sample was stored in a 2.0 mL microcentrifuge tube (Fisher Scientific, Pittsburgh, PA). With the flap of the tube open, the samples were then dried for 4 h at 65°F, after which the tubes were closed and stored in ambient conditions. Samples were analyzed in 96-well plates using a Randomized Complete Block Design including two checks (B101 and B45o2), and six standards consisting of known concentrations of commercially prepared amino acids. The B101 inbred was chosen as a check for its exceptionally high levels of methionine (Hallauer and Wright, 1995). The B45o2 inbred, an *opaque-2* mutant, was used as a check for high tryptophan. The standard concentrations were 5, 20, 35, 60, 75, and 100  $\mu$ M for methionine. To ensure the precision of the assays, the experiment was replicated on three plates (i.e., three blocks). The checks were replicated twice within a plate and the standards three times within a plate. Ten milligrams of each ground sample and checks were weighed into the well of a V-bottom, 96-well microtiter plate.

Microbial methods have been shown to be effective for screening for amino acid levels in grain (Wright and Orman, 1995). We used a microbial analysis method to analyze each

sample as follows. Each sample was subjected to enzymatic hydrolysis using pepsin, a proteolytic enzyme that cleaves peptide bonds indiscriminately. To each well of a conical-bottom 96-well plate (Nunc, Roskilde, DK), 200  $\mu$ L of 0.1 mg/mL pepsin solution in a KCl-HCl pH2 buffer was added. The plate was then sealed, covered with a lid, and placed in a 37°C shaking incubator for approximately 15 h. After the incubation period, the plate was centrifuged at 3000 rpm for 20 min, after which the supernatant was removed for further analysis.

Three different auxotrophic strains of *Escherichia coli* were used to determine methionine, tryptophan, and lysine concentration in the hydrolyzed protein extracts. The inoculum was prepared in M9 media supplemented with 10  $\mu$ L of 1 mg/mL methionine, lysine, or tryptophan solution per 5 mL M9 media (Maniatis et al., 1982) and grown to late log phase. Five (for methionine analysis) or ten (for tryptophan and lysine analysis) microliters of hydrolysate or a standard were transferred directly into a flat-bottom, 96-well assay plate (Corning Incorporated, Corning, NY). The plates were sealed between operations to prevent evaporation. To each well, 100  $\mu$ L of M9 media and 2  $\mu$ L of the inoculum were added. The plate was then sealed, covered with a lid, and placed in a 37°C shaking incubator for 7 h. After the incubation period, the plates were placed on a plate shaker for 3 min and the 595 nm light scattered by the sample was determined using a microplate reader.

## Protocol for Rodent Feeding Trial

The selections made for high and low methionine in the BS11 populations were used to formulate diets that comprised two treatments in the feeding trial. The remnant grain of the bulks selected for the feeding trial was ground using a pin mill located at the Center for Crop Utilization Research at Iowa State University. The maize kernels were ground to an average particle size of 90 U.S. Standard. Forty 1-mo-old female Sprague-Dawley rats were penned individually in wire-floored cages to facilitate the daily collection of any spilled feed, and were acclimated to these conditions for 3 d. During this time, the rats were fed standard corn, which was supplemented with the necessary minerals and vitamins, and water ad libitum. On the fourth day, the 40 rats were weighed and 15 rats of similar weights were randomly allotted to each of two dietary treatments: BS11HM or BS11LM. Again, feed and water were provided ad libitum. In addition to the dietary treatments, the basal diet consisted of minerals, vitamins, soybean meal concentrate, and supplements of the essential amino acids lysine, threonine, and tryptophan (Table 1). Feed intake and body weight gain were measured at 4-d intervals until each rat achieved  $170 \pm 6$  g body weight. The daily consumption was monitored by weighing the feed provided to the rats and subtracting the mass of spilled feed. Feed efficiency was calculated at each weighing day by dividing the mass gain since the last weighing day by the mass of feed consumed since the last weighing day.

## Statistical Analysis

To make mean comparisons of the categories within the different populations and among the two diets in the feeding trial and to obtain the analyses of variance, the GLM procedure was used. The mean methionine concentrations were used in the analysis of variance.

**Table 1. Diet composition for the rat feeding trial with the corn meal from the selections made from the improved BS11 population. Three batches of three kilograms for each diet were prepared from the same recipe to feed the rats for 25 d.**

Ingredient	Experimental diets	
	HM Diet (g)	LM Diet (g)
Corn, High Met	2400.0	–
Corn, Low Met	–	2400.0
Soy concentrate	300.0	300.0
Lysine	1.5	1.5
Threonine	0.6	0.6
Tryptophan	0.7	0.7
Selenium Mix	1.5	1.5
Corn Oil	90.0	90.0
Starch	104.1	104.1
Calcium	69.0	69.0
Limestone	10.2	10.2
Salt	6.0	6.0
Choline Chloride	9.0	9.0
ISU Mineral Mix	1.5	1.5
Base Vitamin Mix	6.0	6.0

To evaluate the feeding trial data, feed efficiency (expressed as weight gain/feed consumed) was analyzed with analysis of covariance using a standard least squares fitting personality. The treatment effect (HM or LM diet) defined as fixed and the day of the study as the regressor:

$$Y_i = \mu + \text{Treatment}_i + \text{Day of the study} + (\text{Treatment} \times \text{Day of the study}) + \text{error}_i$$

where Y is the observed value of the treatment

$\mu$  = overall mean of observed values

$\text{Treatment}_i$  = The effect of the  $i^{\text{th}}$  treatment (HM Diet or LM Diet)

Day of the study = the regressor, the day on which the rats were weighed

$\text{error}_i$  = the error associated with the given treatment

and the remaining terms are interactions of the terms listed above.

To evaluate three cycles of selection for methionine, traits were analyzed with the three cycles of selection for HM designated with positive numbers and the three cycles of selection for LM designated with negative numbers. Thus the cycle effect was a continuous variable ranging from –3 to +3. An analysis of covariance was performed using a standard least squares fitting personality with all effects defined as fixed and cycle as the regressor:

$$Y_{ijk} = \mu + \text{Rep}_i + \text{Year}_j + \text{Pop}_k + \text{Cycle} + (\text{Year} \times \text{Pop}) + (\text{Year} \times \text{Cycle}) + (\text{Pop} \times \text{cycle}) + (\text{Year} \times \text{Pop} \times \text{Cycle}) + \text{error}_{ijk}$$

where Y is the observed value of the treatment

$\mu$  = overall mean of observed values

$\text{Rep}_i$  = the effect of the  $i^{\text{th}}$  rep (rep 1 or rep 2)

Evaluation Year $_j$  = the effect of the  $j^{\text{th}}$  Evaluation year (year 1 or year 2)

$\text{Pop}_k$  = the effect of the  $k^{\text{th}}$  population (BS11 or BS31)

Cycle = the effect of the regressor: selection cycle (–3 through +3)

$\text{error}_{ijkl}$  = the error associated with the given treatment

and the remaining terms are interactions of the terms listed above.

Amino acid analysis of the Cycle 3 samples was analyzed by fitting the following fixed effects model with a standard least squares personality:

$$Y_{ijkl} = \mu + \text{Rep}_i + \text{Sel}_j + \text{Pop}_k + \text{Year}_l + (\text{Sel} \times \text{Pop}) + (\text{Pop} \times \text{Year}) + (\text{Sel} \times \text{Year}) + (\text{Sel} \times \text{Pop} \times \text{Year}) + \text{error}_{ijkl}$$

where Y is the measured value of each amino acid

$\mu$  = overall mean of observed values

Sel = the effect of the  $j^{\text{th}}$  selection (HM or LM)

Pop = the effect of the  $k^{\text{th}}$  population (BS11 or BS31) and

Year = the effect of the  $j^{\text{th}}$  year (evaluation year 1 or evaluation year 2)

$\text{error}_{ijkl}$  = the error associated with the given treatment

and the remaining terms are interactions of the main effects listed above.

## RESULTS

### Selection for Grain Methionine Concentration to Produce Grain for a Feeding Trial

To produce sufficient levels of grain for a feeding trial while carrying out divergent selection for grain methionine concentration, we first evaluated  $F_1$  ears from two random mated populations, BS11 and BS31. Five ears with the highest methionine concentration were selected from each population to form two sub-populations designated BS11HM and BS31HM. Similarly, the five ears with the lowest methionine concentration were selected to form the populations BS11LM and BS31LM. We initiated a breeding program by planting each selection ear-to-row and intermating within each selection. The methionine concentration of the ears resulting from each selection was then analyzed, and the mean methionine values for each category are reported in Table 2. In BS11, the mean methionine concentration of the population derived from HM was significantly higher than that for LM at the  $P < 0.05$  level. In BS31, the mean methionine concentration of the population derived from HM selections was significantly higher than the mean methionine concentration of the population derived from LM selections at the  $P < 0.05$  level. The differences between the means of the methionine concentration of the High and the Low categories of BS11 were larger than the difference in BS31. BS11HM contained 23% more methionine than BS11LM, and this difference was statistically significant ( $P = 0.05$ ).

To generate the approximately 10 kg of grain required for the trial, it was necessary to grow the selections for another generation. Selected ears were planted ear-to-row and plants within each row were sib-mated and harvested in bulk. We then analyzed the composition of bulks from



**Table 2. Evaluation of methionine concentration for each category of populations derived from BS11 and BS31 for the feeding trial experiment. All statistically significant differences show that the mean value of the High category is higher than the mean value of the Low category.**

Population	Selection <sup>†</sup>		Contrast
	HM	LM	
BS11	0.179	0.168	**
BS31	0.204	0.193	**

<sup>†</sup>Relative values for the trait represented by the optical density measurement corrected for the mass of the sample.

\*\*Significant at  $P < 0.05$ .

BS11HM and BS11LM in greater detail to design an appropriate feeding trial. The three bulks from BS11HM with the highest methionine concentration were bulked to form one HM batch and the three bulks from BS11LM with the lowest methionine concentration were bulked to form one LM batch. To confirm the effectiveness of the selection protocol and to further characterize the selections, the complete amino acid composition of these batches was determined (Table 3). This complete amino acid composition provides additional information regarding the effect of selection for methionine on the other amino acids. Of the amino acids analyzed, methionine differed the most between the HM and LM populations. Cysteine is also of interest because it is the other sulfur-containing amino acid. It was more different than most other amino acids between the HM and LM populations as well. Taken together, the ratio of the sulfur-containing amino acids in HM to that in LM is 0.51 to 0.42, a 17.6% difference. Overall, there was an increase in total protein and in most other amino acids when selecting for HM.

To further characterize this material, NIR spectroscopy was used to predict the total protein, oil, and starch concentration of each class (Table 4). Starch and protein were found to be significantly different between the HM and the LM categories, with the LM population having more starch and the HM population having more protein. Significant differences were not observed between the High and the Low category for oil.

## Rat Feeding Trial with Grain Selected for High or Low Methionine Concentration

Having established that significant differences exist in amino acid composition between the HM and LM grain from BS11, we next compared these entries in a rat feeding trial to determine if the measured changes resulted in an altered nutritional quality. The grain was supplemented to formulate two diets that were not limited in lysine, threonine, or tryptophan as summarized in Table 1. A total of 7.5 kg for each diet were prepared for the feeding trial and stored in a refrigerator until further use. Rats were weighed and feed consumption was calculated on the first day of the trial and at 4-d intervals thereafter.

**Table 3. Complete amino acid profile obtained using the AOAC standard method for the grain of the feeding trial entries. All values are given in g of amino acid per 100 g of tissue.**

Amino Acid	HM	LM	HM/LM
Taurine	0.00	0.00	0.00
Hydroxyproline	0.02	0.02	0.00
Aspartic Acid	0.65	0.65	0.00
Threonine	0.34	0.32	1.06
Serine	0.40	0.39	1.03
Glutamic Acid	2.15	2.03	1.05
Proline	0.97	0.91	1.07
Lanthionine	0.01	0.01	0.00
Glycine	0.38	0.35	1.09
Alanine	0.84	0.79	1.06
Cysteine	0.25	0.22	1.14
Valine	0.53	0.51	1.04
Methionine	0.26	0.20	1.30
Isoleucine	0.38	0.37	1.02
Leucine	1.43	1.35	1.06
Tyrosine	0.30	0.30	0.00
Phenylalanine	0.54	0.53	1.02
Hydroxylysine	0.00	0.00	0.00
Histidine	0.30	0.29	1.03
Ornithine	0.01	0.01	0.00
Lysine	0.29	0.27	1.07
Arginine	0.45	0.44	1.02
Tryptophan	0.07	0.06	1.17
Total	10.57	10.02	1.05

The treatment effect estimate of 0.0091 (Table 5) indicates that the HM diet had a greater efficiency than the LM diet by 0.018 ( $= 2 \times$  the treatment effect) g of gain/g of feed consumed, (Table 5). The feed efficiency decreased as the study progressed, but the lack of a significant Treatment  $\times$  Day of Study interaction indicates that the difference between treatments did not change significantly over the course of the study. The difference in feed efficiency was driven by a difference in feed consumption early in the study, with rats on the HM diet consuming less feed than rats on the LM diet.

**Table 4. Near-Infrared (NIR) spectroscopy analysis for the grain of the feeding trial entries, expressed as percent, adjusted to 0% moisture and corrected for bias.**

Trait	Population		Contrast
	BS11HM	BS11LM	
Protein	13.9	12.6	*
Oil	5.1	5.0	NS <sup>†</sup>
Starch	66.9	68.9	***

\*Significant at  $P < 0.10$ .

\*\*\*Significant at  $P < 0.005$ .

<sup>†</sup>NS, not significant.

**Table 5. Analysis of the rat feeding trial data by ANOVA using a fixed effects model with a covariate.**

Trait	Effect <sup>†</sup>			Model R <sup>2</sup>
	Treatment	Day of study <sup>‡</sup>	Pop x Day of study	
Gain/feed	0.0091	-0.016	NS	0.83

<sup>†</sup>NS indicates the effect was not significant. If the effect was significant at  $P < 0.05$ , then the estimate of the magnitude of the effect is listed with a greater HM treatment being expressed as a positive value.

<sup>‡</sup>Day of study is fitted as a linear covariate, so the slope is given.

## Evaluation of Three Cycles of Recurrent Selection for Methionine Concentration

While intermating within selections was effective for producing differentiated grain in sufficient amounts for a feeding trial, this process would be predicted to result in a rapid loss of genetic diversity in the populations and may not be suitable for a long-term breeding program. We therefore initiated a second breeding program that involved random intermating among selections. This program started with the same selections as the program used to generate the feeding trial diets but in each cycle selected ears were used to make a balanced bulk that was planted the following year. Intermating was performed among the resulting plants, the resulting ears were evaluated and the best (highest or lowest methionine concentration, depending on selection goal) were selected and used to create a bulk for the next cycle.

Three cycles of selection for and against methionine concentration in BS11 and BS31 resulted in 12 populations (2 starting populations  $\times$  3 cycles  $\times$  HM or LM). To determine the effect of selection for and against methionine concentration, we grew and evaluated these twelve

populations in two reps, each of two ears. Plant height, ear height, silk date, and pollen date were measured. Grain was harvested and average kernel weight, %N, protein, oil, starch, methionine, tryptophan, and lysine were measured. Table 6 summarizes the results of these analyses. The Cycle effect is an indicator of response to selection. Methionine concentration increased significantly by 0.004 g methionine/100 g tissue per cycle (Table 6, Fig. 1). While there was a significant difference in methionine concentration between the BS11 and the BS31 populations, the lack of a significant Pop  $\times$  Cycle effect indicates that the two populations did not respond to selection differently. Several other traits showed significant responses to selection as well, including plant height, ear height, days to silking, and days to pollen. Unlike methionine concentration, however, these traits all had significant Pop  $\times$  Cycle effects, indicating that these changes were different in BS11 and BS31. Kernel mass and composition did not vary significantly with cycle of selection.

To confirm the amino acid measurements made using our microbial method and to obtain additional data on other amino acids, we submitted the samples from the third cycle of selection (HM and LM) to an independent laboratory for amino acid analysis by the AOAC standard method. The results of this analysis are presented in Table 7. A significant response to selection would be indicated by a significant selection effect in this experiment. Of the 12 amino acids analyzed, the only one with a significant selection effect was methionine, with the HM samples having on average 0.014 g/100 g more methionine than the LM population. Only the sulfur containing amino acids cysteine and

**Table 6. Analysis of variance of agronomic and grain composition traits in three cycles of selection for and against methionine concentration.**

Trait (units)	Effect <sup>†</sup>							Model R <sup>2</sup>
	Cycle (Slope) <sup>‡</sup>	Pop (BS11 or BS31)	Year (1 or 2)	Pop $\times$ Cycle	Year $\times$ Pop	Year $\times$ Cycle	Year $\times$ Pop $\times$ Cycle	
Plant Height (cm)	-2.37	NS	29.94	-1.98	NS	NS	NS	0.91
Ear Height (cm)	-2.37	NS	4.74	-1.28	NS	NS	NS	0.61
Days to silking	-0.2	-0.1	4	-0.4	-0.3	NS	NS	0.97
Days to pollen	-0.2	-1	2	-0.5	NS	NS	NS	0.86
Kernel mass (mg)	NS	NS	-9.67	NS	-6.35	NS	NS	0.41
%N <sup>§</sup>	NS	NS	-0.095	NS	NS	NS	NS	0.66
Oil (%dm) <sup>¶</sup>	NS	0.564	NS	NS	NS	NS	NS	0.28
Protein (%dm) <sup>¶</sup>	NS	NS	-0.733	NS	NS	NS	0.22	0.65
Starch (%dm) <sup>¶</sup>	NS	-0.863	0.849	NS	NS	NS	NS	0.39
Met (g/100g) <sup>#</sup>	0.004	-0.013	-0.012	n.s	0.003	NS	NS	0.89
Trp (g/100g) <sup>#</sup>	NS	NS	-0.008	NS	NS	NS	NS	0.50
Lys (g/100g) <sup>#</sup>	NS	-0.013	NS	-0.003	NS	-0.003	NS	0.42

<sup>†</sup>Estimate of the magnitude of the effect is listed with units given in row heading. Positive values indicate the first of the parameters listed in the column heading is greater. When effects are significant ( $P < 0.05$ ) the estimate of the magnitude of the effect is listed. NS indicates the effect was not significant.

<sup>‡</sup>The slope of the regression line of cycle (-3 to +3) vs. trait value. Units are trait units/cycle, for example the units for plant height are cm/cycle.

<sup>§</sup>Determined by combustion analysis.

<sup>¶</sup>Determined by NIR.

<sup>#</sup>Determined by microbial amino acid assay.

methionine had significant “population” effects, illustrating differences in sulfur amino acid concentration between BS11- and BS31-derived populations.

Since the Cycle 3 populations were analyzed by both the microbial method used for selection and the AOAC standard method for methionine analysis, we were able to compare the performance of the microbial method to the standard method. Figure 2 illustrates the correlation between the two methods. The correlation coefficient of 0.94 indicates that there is good agreement in the way the two methods rank samples, however the microbial method produces lower values than the standard method.

### DISCUSSION

The objective of this work was to determine the impact of recurrent selection for methionine concentration in random-mated maize populations. While HM is the trait with the greatest economic interest, we performed selection for LM in parallel to selection for HM to maximize the contrast between populations being compared in this study. The impact of selection on grain nutritional quality was evaluated in a rat feeding study while the impact on plant and grain traits was evaluated by using a replicated field trial. While both experiments involved three generations of divergent selection for methionine concentration, these two experiments required slightly different breeding approaches, and the results of the two experiments are therefore not expected to be the same. In the feeding trial experiment it was necessary to obtain a large amount of grain. Therefore, we selected within families as we bulked grain while advancing through generations. We considered the field evaluation experiment to be a longer-term experiment so we therefore intermated among families to maximize the amount of genetic diversity retained in each generation.

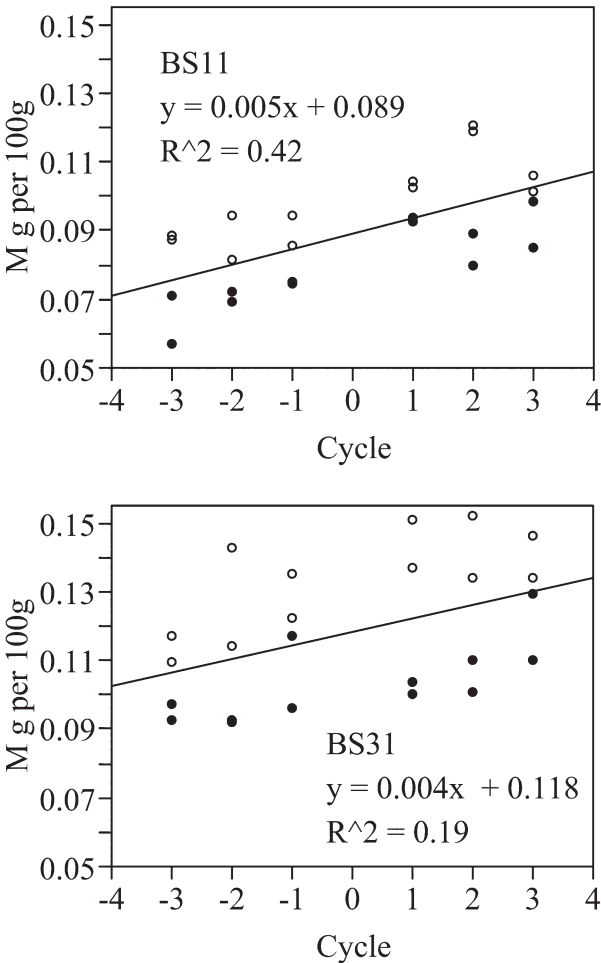


Figure 1. Grain methionine levels in each cycle of selection evaluated by the microbial assay. Open and closed circles represent different evaluation years. LM populations are indicated by negative cycle numbers and HM populations are indicated by positive cycle numbers.

Table 7. Estimates of the effects from a fixed effects model on amino acid concentration in the cycle three grain samples determined using the AOAC standard method for amino acid analysis.

Amino acid	Effect							Model $R^2$
	Selection (HM or LM)	Pop (BS11 or BS31)	Year (1 or 2)	Year x Pop	Year x Selection	Pop x Selection	Year x Pop x Selection	
Asp	NS†	NS	−0.026	NS	NS	NS	0.016	0.74
Thr	NS	NS	−0.010	NS	NS	NS	NS	0.64
Glu	NS	NS	−0.091	NS	NS	NS	0.059	0.73
Pro	NS	NS	−0.024	NS	NS	NS	n.s	0.63
Gly	NS	NS	−0.013	NS	NS	NS	0.008	0.80
Ala	NS	NS	−0.036	NS	NS	NS	0.022	0.75
Cys	NS	−0.006	−0.008	NS	NS	NS	0.004	0.87
Val	NS	NS	−0.020	NS	NS	NS	0.010	0.78
Met	0.014	−0.015	−0.010	NS	NS	NS	NS	0.91
Ile	NS	NS	−0.016	NS	NS	NS	NS	0.74
Leu	NS	NS	−0.066	NS	NS	NS	NS	0.72
Lys	NS	NS	−0.006	NS	NS	NS	0.006	0.68

†When effects are significant ( $P = 0.05$ ) the estimate of the magnitude of the effect is listed in g/100g tissue. Positive values indicate the first of the parameters listed in parentheses is greater. NS indicates the effect was not significant.

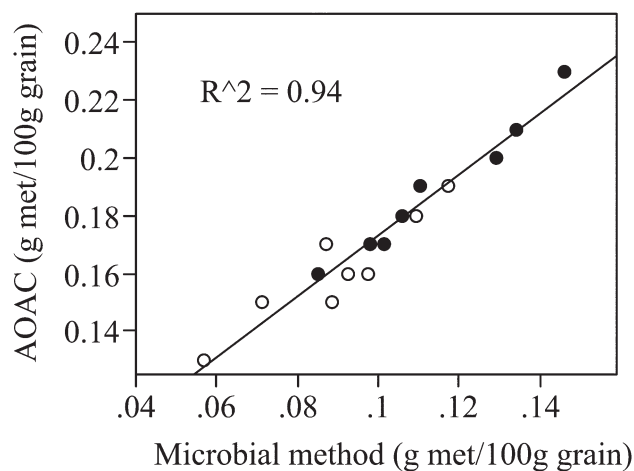


Figure 2. Correlation between the microbial method used for selection and the AOAC standard method for methionine analysis. Samples were from Cycle 3 of BS11HM, BS11LM, BS31HM, and BS31LM populations produced in two years with two field reps in each year. Open circles represent low methionine populations and filled circles represent high methionine populations.

### High Methionine Grain Has Higher Feed Efficiency than Low Methionine Grain

To determine if selection for methionine concentration increased the nutritional value of the populations under selection, we conducted a rat feeding trial comparing the feed efficiency of diets containing corn selected for HM or LM. Rats fed the diet based on the HM population had significantly higher feed efficiency than those on the LM diet. In other feeding trials conducted with monogastric animals, the response to supplemental methionine has been inconsistent (Russell et al., 1986). In only one of seven treatments, feed efficiency of growing pigs fed maize-soybean meal diets was improved when methionine was the only amino acid added to the diet (Russell et al., 1986). In chickens fed a low protein diet of soybean and herring meal, it was reported that the feed consumption was reduced and that the feed efficiency was not affected when compared to the control (Fockede and Arnould, 1978).

The experimental maize diets differed not only in methionine concentrations but also in other amino acids and in the total protein concentration. The total nitrogen concentration of the complete diets was higher for the HM diet than for the LM diet. Thus, the total protein available in the diets was not altered with the supplementation of the soybean meal. The protein concentration in the HM diet remained higher than the protein concentration in the LM diet, regardless of the other sources of protein. The percent moisture was not significantly different between the diets. It is therefore possible that the rats fed on the LM diet consumed more feed to meet the dietary demands for protein than the rats fed on the HM diet, but it is not clear if this difference is due to a difference in methionine content or to a difference in the total protein content of the diet.

### Evaluation of Three Cycles of Recurrent Selection for Methionine Concentration

The effect of selection for methionine concentration was evaluated by growing three cycles of selection for high or low methionine concentration in two populations in field trials and evaluating plant and grain traits. Overall, methionine concentration responded positively to selection. The rate of gain was not large, but was sufficient to generate significant differences between the methionine concentration of the high and low populations after three cycles of selection (Table 7). It may be possible to increase the rate of gain by increasing the selection pressure from the approximately 10% level used in this experiment.

The precision of the effect of selection for methionine was remarkable. Methionine was the only amino acid analyzed that showed evidence of response to selection. Even amino acids sharing the same biosynthetic pathways as methionine such as cysteine, lysine, and tryptophan showed no evidence of selection in the field trial experiment. Total nitrogen percentage and protein concentration remained unchanged by selection in this experiment as well, although in the feeding trial experiment the HM corn contained more protein than the LM corn. Choe et al. (1976) found that total protein concentration increased in the course of selection for lysine. While several agronomic traits changed in response to selection, they changed to different degrees in BS11 and BS31, which makes it likely that this is a consequence of genetic drift, rather than an effect correlated with the response to selection for methionine.

There is some discrepancy in the results of the microbial analysis method that was used for selection and the AOAC standard method for methionine analysis, with the microbial method producing much lower values than the standard method. This may be because the pepsin digestion used in the microbial assay give a less efficient hydrolysis than the acid hydrolysis used in the standard method. For making selections in a breeding program, the ability to rank samples repeatably is more important than producing accurate values. The high correlation between the microbial method and the AOAC standard method suggests that the microbial method should be an effective selection tool. This idea is validated by the observation that we made gains that were measureable with the standard method using the microbial method as a selection tool.

### CONCLUSIONS

We have examined the impact of direct selection for grain methionine concentration and determined it is possible to alter grain methionine concentration with this approach. The specificity of the alteration was remarkable. Comparing HM and LM populations, methionine concentration was the trait exhibiting the largest and most consistent difference. The HM population had a higher feed efficiency in a rat feeding trial than the LM population. This may



be an attractive approach for improving grain quality in some cases. In addition, these populations may prove to be valuable for increasing our understanding of the genetic control of grain methionine concentration. Because the HM and LM population pairs are derived from the same starting population and differ significantly in methionine concentration, they could be useful for identifying genes controlling this trait.

## Acknowledgments

We thank Merinda Struthers, Rich Clayton, and Chase Lucas for technical support and Marie Hasenstein and Adrienne Moran Lauter for critically reviewing this manuscript. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. This manuscript is a joint publication of the USDA-ARS Corn Insects and Crop Genetics Research Unit and the Iowa Agriculture and Home Economics Experiment Station.

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